the hydrolyzed solution had a specific rotation of $-14.7°$. From this same hydrolyzed material an osazone was obtained and after recrystallization a melting point of 212° C. was determined.

There are three known osazones having this melting point, namely, glucosazone, fructosazone, mannosazone. Attempts to obtain saccharic acid from the sugar were unsuccessful. A ketose was indicated by a positive Seliwanoff reaction, however, no osazone could be obtained using methyl phenylhydrazine (7).

The presence of a glucoside has been definitely established but its composition has not yet been determined. Work is to be carried on in this direction during the year 1933.

BIBLIOGRAPHY.

(1) "Index Kewensis."
(2) "Trans. Am. Phil. Soc. N. S.," 7 (1841), 313.
(3) *Ibid.*
(4) "Bull. Torr. Club," 18 (1891), 225.
(5) Jenkins and DuMez, "Quant. Pharm. Chem.," page 229.
(6) U. S. P. X, page 465.
(7) Armstrong, "The Simple Carbohydrates and Glucosides," 4th Edition, page 96.

LABORATORY OF THE SCHOOL OF PHARMACY,
UNIVERSITY OF OKLAHOMA,
NORMAN, OKLAHOMA.

THE STORY OF THE ISOLATION OF CRYSTALLINE VITAMIN D_1.

A REVIEW ARTICLE BY K. JOSEPHSON IN *Farm. Revy*, 30 (1931), 566–571.

TRANSLATED BY C. S. LEONARD.

Translator's Note: That Windaus should have been successful in isolating crystalline vitamin D_1 is not surprising. Sterol chemistry has occupied his attention since his early teaching days. His *Habilitationschrift* as Privat Dozent at Freiburg in 1903 was on the subject: Cholesterol. Indeed Freiburg had a tradition in the field, for van Oordt in 1901 under Kiliani's tutelage presented a doctor's thesis from Gatterman's laboratory on cholesterol, as did, in 1905, Windaus' pupil, Gustav Stein. Meanwhile, Windaus began publishing a series of articles in the *Berichte* on the chemistry of this sterol, which led to partial formulation of the structure in 1908. A pupil, Adamla, in 1911 wrote a further dissertation on the subject and again in 1919, 1923 and 1925, Windaus published work on phytosterols. So the important phytosterol, ergosterol, was but one member of a family familiar to this chemist. He began to report on the study in 1923. It was but natural that he should become interested in the subject of isolation of pure crystalline vitamin when the occurrence of the D vitamin had been traced to the sterol portion of the unsaponifiable fraction of fats. This sterol fraction is largely cholesterol and Hess and Weinstock had shown that cholesterol could be activated by light but *not* if pure. The patience and skill which won a Nobel prize is recorded below:

In volume 489 of *Liebig's Annalen* (page 252) Windaus reports that in his work upon ergosterol irradiated with ultraviolet light he has succeeded in obtaining a crystalline vitamin which he calls vitamin D_1. As such a result in the field of vitamin research so intensively followed in recent years ought to interest pharmaceutical workers, a summary of the more essential findings is given.

In January 1927, Windaus (with Hess) determined that ergosterol on irradiation with ultraviolet light forms quite an active antirachitic substance, the D vitamin. From that time an intensive study went on in Windaus' laboratory for the isolation of the vitamin in pure crystalline

form. In the work now published (1931), Windaus summarizes the many researches in various directions made to gain the end sought.

As a first step he and his colleagues found that with long-continued irradiation ergosterol wholly decomposed, and also that with continued irradiation the D vitamin formed likewise disappeared. Of great practical importance was the discovery that all the photochemical reaction products arising on irradiation, in contrast to the unchanged ergosterol, were not precipitable with digitonin, and hence could be separated quantitatively from the unchanged ergosterol. On the basis of properties displayed by the irradiation products, in particular from their optical properties, it was concluded that the photochemical reaction of conversion initiated by the irradiation led to many products and that from the amorphous resin formed no crystalline product could be separated although many techniques were used.

So they turned to another method of approach. *First*, the action of visible light in the presence of a sensitizer such as eosin was studied. Wholly inactive products were obtained. Even attempts to activate in a thermal way, heating to 225–300°, were in vain. This was scarcely surprising for it has since been found that even heating to lower temperatures than the above destroys D vitamin. In the attempts to gain new reaction products by the aid of well-defined chemical reactions such as oxidation, hydrogenation, dehydrogenation, dehydration and isomerization, many new crystalline compounds were obtained which doubtless were of interest for increasing our knowledge of the chemistry of ergosterol but appeared useless for solving the actual vitamin question, for not one of them, either before or after irradiation, displayed any D vitamin activity.

On the basis of these negative results they went back to the study of ultraviolet irradiation of ergosterol. It was observed that the presence or absence of oxygen in the irradiation experiments was of great importance as regards the composition of the reaction products. Analytical studies of the products obtained, almost always showed that they were richer in oxygen than the starting materials in case oxygen had been present in the experiment.

Since it might be only the presence of such oxygen richer compounds which hindered crystallization of D vitamin, it seemed worth while to investigate the possibiity of getting positive results by a full exclusion of oxygen both in irradiating and in the after treatment of the reaction products during the isolation of the vitamin. So they worked in an atmosphere of oxygen-free CO_2 or in a high vacuum. In this way they got a product with about a 60% photochemical conversion of the ergosterol, which showed a high D vitamin action and was completely free from oxidation products. But here also no crystalline substance could be isolated. The product consisted of a mixture of ergosterol isomers. On the basis of its behavior to various reagents it was concluded that the vitamin must contain a system of conjugated double bonds and also an hydroxyl group. On distillation in high vacuum the substance lost most of its antirachitic activity. (This is noteworthy for Bourdillon later succeeded in isolating in this manner a substance called *Calciferol* which crystallizes readily and is strongly antirachitic.) According to Windaus no other physical method of separation of many tried led to a crystalline substance.

Since this type of experiment failed to yield pure vitamin they next tried to make crystalline derivatives of the vitamin. Many esters of the irradiation products were prepared; not one of these gave the desired result. But Windaus made the interesting observation that the esters of the vitamin with acetic acid and with palmitic acid were physiologically active while esters of acids not normally occurring in the body possessed no antirachitic activity.

As studies with the non-oxidized 60% conversion product were a failure, finally the product resulting from short irradiation periods was studied. On the assumption that, in the first photochemical change of ergosterol, only one reaction product is formed and that this is identical with the vitamin, it might be possible to isolate the vitamin from short-term irradiation products of about 25% conversion for probably only unchanged ergosterol precipitable by digitonin would be mixed with the vitamin. Yet no crystalline substance was obtained in this way. The preparation obtained did not appear essentially different from preparations got by medium-long irradiation periods. So it is likely that even in the first photochemical change of ergosterol many reaction products are formed.

Now consideration was given to irradiation with monochromatic light or at least with filtered radiation, hoping thereby to arrive at a single reaction product. In these experiments they used (1) unfiltered mercury arc light, (2) light from a magnesium spark with maximum in-

tensity between 278 and 280 mμ and a weak intensity below 277 mμ and in the region 295 to 313 mμ, (3) mercury arc light filtered through uviol glass double filters passing only wave-lengths above 290 mμ, (4) the mercury arc light filtered through a salicylic acid solution which absorbed the region from 275–325 mμ. The interesting discovery was then made that preparations of 50% converted ergosterol made by aid of the various light sources could scarcely be distinguished one from another. Yet there was a great difference in the optical rotatory power and in this way it was determined that the longer the wave-length of ultraviolet light used the stronger the positive rotation. However, no product could be got out in a well-crystalline state.

While this work was going on in the laboratory of Windaus, an article was published by Reerink and van Wyk in which it was claimed that with ultraviolet light of greater wave-length than 275 mμ a very simple photo-chemical reaction occurred with formation of a product about 60% converted, yielding a nearly pure vitamin which could be obtained in crystalline condition but had a melting point below 0° C. Use of shorter wave-lengths of light they said led to a more complicated photo-chemical reaction. Testing out these results in Windaus' laboratory, certain preparations were obtained with an antirachitic protective dose of about the same order as those of previously studied preparations obtained by irradiation with various light sources but no preparations had as homogeneous activity as preparations such as those studied by Reerink and van Wyk should have shown.

At that time the use of filter irradiation for photo-chemical conversion of ergosterol to vitamin D by others aroused lively interest (Steenbock; Rosenheim and Webster; Bills). In 1928 the observation had been made that large doses of ergosterol are toxic and that this toxic action, besides loss of weight, also was manifested by calcification in various organs (Kreitmann; Klein; Smith and Elvolve; and others). A controversy arose on whether or not the therapeutic and the toxic action originated from one and the same factor or from two different factors occurring side by side in the irradiation products. Later, Heuber and also Schuenert and Schielen found that the two activities were fully parallel in commercial preparations and hence no ground could be seen for the view that the two effects were ascribed to different factors. Meanwhile Reiter (1929) and Kisch and Reiter (1930) reported that if ergosterol is irradiated with light of greater wave-length than 280 mμ a highly active antirachitic preparation results, which in contrast to the preparations made with unfiltered light, displayed no toxic action. These results, which awakened great interest, were tested by Windaus who could not confirm them. The question remained unanswered as to whether or not the antirachitic and toxic action arose from one and the same or from two different chemical individuals. Windaus next found, and his colleagues confirmed the fact a little later, that irradiation products which were treated with sodium and alcohol or which were heated to 200°, no longer possessed antirachitic activity but did show the toxic effect. If both activities were to be ascribed to one and the same substance, it must be concluded that by chemical reaction this substance could lose one activity without loss of the other. If two different factors were at the basis of the two different effects, the antirachitic substance was definitely less stable than the toxic one. Solution of the question would only be possible if one could succeed in separating the two factors from one another without losing the activity of either.

Attempts to separate the two factors were then begun in Windaus' laboratory and they used maleic acid anhydride and citraconic acid anhydride which the studies of Diels and Alder had shown can be added to conjugate double bonds. These reagents had already been used by Windaus in his experiments for determining the position of the double bonds in ergosterol derivatives and he had worked out a method for quantitative differentiation of various ergosterol isomers. With the action of maleic anhydride on the irradiation products of ergosterol the following results were obtained: The preparation, which has been 60% converted by irradiation with the unfiltered magnesium spark, reacted comparatively rapidly with maleic anhydride and gave a high yield of the compound with it. The product obtained with the aid of xylol-filtered magnesium spark light reacted more slowly and less fully. The preparation made with unfiltered mercury arc light likewise reacted weaker than the magnesium light preparations. The preparation made by irradiation with light filtered through the double uviol glass filter reacted with the reagent the slowest and least. It should be noted that this light was of the longest wave-lengths of those employed. Now they found that the fractions of the irradiated preparations which did not react with maleic anhydride had very constant physical properties. Thus, these fractions somewhat absorbed the spectrum in the short wave region and the optical rotation had become

positive. The antirachitic potency and also the toxic action were of full normal value and no separation of the two factors had been brought about in this way. But it was of special importance that these preparations on standing in petroleum-ether solution *gradually crystallized nearly completely.* Later they found that even preparations which had not been treated with maleic or citraconic anhydride could be brought to crystallize by seeding them with crystals obtained in the above manner. The treatment with one or the other of the anhydrides has shown itself, however, to be the best technique yet found for preparing crystalline vitamin D_1. The designation D_1 is on account of the probability that many bodies present in irradiated ergosterol possess antirachitic activity.

The following example shows how the preparation of crystalline vitamin D_1 was conducted: Eight grams of ergosterol were dissolved in 270 cc. of absolute ether and irradiated in a quartz vessel by mercury arc light for nine hours. The conversion of ergosterol amounted to 59%. The irradiation products freed from ergosterol showed the specific rotation +26.8°. 3.4 grams of the irradiation product were let stand in ether solution for ten days at room temperature with 3 Gm. of citraconic anhydride. After this the material was separated into a neutral and acid fraction. 55% of the irradiation product had bound citraconic anhydride. The neutral part (1.58 Gm.) crystallized almost completely. By recrystallization from acetone 0.61 Gm. of pure vitamin D_1 was obtained as long, well-formed needles of melting point 124–125°. In a high vacuum this distills with decomposition at 135°; on heating to 180° it decomposes. The optical rotation (Na light) was +140.5° in acetone and alcohol. Vitamin D_1 combines faster with maleic anhydride than with citraconic anhydride. The reactions indicate that a system of conjugate double bonds exist in the molecule. By analysis and determination of molecular weight vitamin D_1 was shown to be an isomer of ergosterol.

Of great interest is the physiological behavior of vitamin D_1. It is worthy of note that the antirachitic activity of the crystalline D_1 is about three times weaker (dose 0.03γ) than that reported by Reerink and van Wyk for their preparation. The relationship of antirachitic and toxic effect remains the same as in non-crystalline preparations. Windaus considers that to be evidence that the antirachitic vitamin itself is the cause of the toxic effect.

Bourdillon's "Calciferol" is like Windaus' D_1 in properties except that it has far higher rotatory power. Windaus thinks it is a mixture.[1]

O. Linsert (a pupil of Windaus) of the I. G. laboratories in Elberfeld has reported (after Windaus' description of vitamin D_1 went to press) isolation of a vitamin D_2 from the irradiation products of ergosterol. D_2 vitamin melts at 114–115° [later given 115–116°] and has a specific rotation of +85° [later +82.6°]. It is more antirachitic than D_1 and displays the usual toxic effect. [0.02 and 0.015γ doses were protective.]

Further Developments —Some of the above can now be obtained in English from Windaus' own hand in *Proc. Roy. Soc.*, London, 108B (1931), 568–575. Windaus has since shown that vitamin D_2 is a component of D_1 which latter consists of a monomolecular addition compound of D_2 with an isomeric physiologically inactive alcohol, *lumisterin.* When molecular equivalents of lumisterin and vitamin D_2 are mixed, the addition compound has the physical constants of vitamin D_1. The D_1 melting point, 124°, is higher than those of the components. Lumisterin is nontoxic as well as non-antirachitic. Lumisterin melts at 118° and has a specific rotation of +191° in acetone. On irradiation with unfiltered magnesium spark light it is converted to Vitamin D_2. It appears to be an intermediate product on the way from ergosterol to Vitamin D_2 which latter may thus be looked upon as the true pure vitamin D. (*Ann.*, 492 (1932), 226; 493 (1932), 259.)

[1] *Translator's Note:* Bourdillon and co-workers first described in 1930 separation by distillation at 145° C. in high vacuum of a crystalline highly antirachitic substance melting after recrystallization from alcohol at 113–115°. In 1931 they named as *Calciferol* a product so obtained, melting at 123–125° with specific rotation of +260° in alcohol (5641 line). Later, that year, they showed that the calciferol previously described consisted to the extent of 30–50% of an inactive *Pyrocalciferol* and that pure calciferol resembled vitamin D_3 rather than D_1. Pure calciferol is described (1932) as possessing twice the antirachitic potency previously recorded for calciferol and to be of m. p. 114.5–117° with a specific rotation of +102.5° in alcohol (Na light). The purified product is obtained by removal of unchanged ergosterol with digitonin, treatment with 3,5-dinitrobenzoyl chloride, separation of the dinitrobenzoate of calciferol and hydrolysis of the latter. It appears that the early calciferol was a mixture of the molecular addition compounds of the vitamin with lumisterin and pyrocalciferol. Thus Bourdillon's group appear to have confirmed Windaus on many points and to have discovered independent methods of isolation of the vitamin. See *Proc. Roy. Soc.*, 107B (1930), 76; 108B (1931), 340; 109B (1932), 488; *Nature*, 128 (1931), 758.